MATURE AIRWAY ORGANOIDS, METHODS OF MAKING AND USES THEREOF

FIELD OF THE INVENTION

[0001] The invention is generally directed to airway organoids, particularly differentiated airway organoids, methods of making and using, particularly for influenza virus research.

BACKGROUND OF THE INVENTION

[0002] Influenza A viruses (IAVs) can infect a diversity of avian and mammalian species including humans, and have the remarkable capacity to evolve and adapt to new hosts (1). The segmented RNA genomes of IAVs and the low fidelity of RNA polymerase allow for antigenic shift and drift, which drive this evolution. Thus, novel viruses from birds and pigs can cross the species barrier and infect humans, leading to sporadic infections, epidemics and even pandemics (Klenk, *Cell Host Microbe* 15(6):653-654 (2014); To, et al., *Lancet* 381(9881):1916-1925 (2013)). Despite the tremendous progress made in virology and epidemiology, it remains unpredictable which subtype or strain of IAV will cause the next outbreak. A novel reassortant H7N9 influenza virus from poultry has led to recurrent outbreaks of human infections in China since 2013 (To, et al., *Lancet* 381(9881):1916-1925 (2013)), Chen, et al., *Lancet* 381(9881):1916-1925 (2013)). According to a World Organization report more than 1500 laboratory-confirmed cases of H7N9 human infections were reported by October 2017, with a case-fatality rate higher than 35%. In 2009, the first influenza pandemic of the 21st century was caused by a novel pandemic H1N1 (H1N1pdm), which originated via multiple reassortment of "classical" swine H1N1 virus with human H3N2 virus, avian virus and avian-like swine virus (AVIT, et al., *N Engl J Med* 360(25):2605-2615 (2009)). While swine viruses only sporadically infect humans, this novel strain of swine-derived H1N1pdm virus can establish sustained human-to-human transmission and has been circulating globally as a seasonal virus strain since then. Proteolytic cleavage of viral glycoprotein hemagglutinin (HA) is essential for IAV to acquire infectivity since only the cleaved HA molecule mediates the membrane fusion between virus and host cell, a process required for the initiation of infection. HA proteins of low pathogenic avian IAVs and human IAVs carry a single basic amino acid arginine at the cleavage site (Bottcher E, et al., *J Virol* 80(19):9896-9898 (2006); Bosch, et al., *Virology* 113(2): 725-735 (1981)), recognized by trypsin-like serine proteases. Productive infection of these viruses in human airway thus requires serine proteases like TMPRSS2, TMPRSS4, HAT etc. (Bottcher-Friebertshauser et al., *Pathog Dis* 69(2):87-100 (2013). However, HA proteins of high pathogenic avian viruses, such as H5N1, contain a polybasic cleavage site that is activated by ubiquitously expressed proteases.

[0003] Current in vitro models for studying influenza infection in human respiratory tract involve short-term cultures of human lung explant and primary airway epithelial cells. Human lung explants are not readily available on a routine basis. In addition, rapid deterioration of primary tissue in infection experiments is a major problem. Under air-liquid interface conditions, basal cells isolated from human airway can polarize and undergo mucociliary differentiation. Yet, this capacity is lost within 2-3 passages (Butler, et al., Am J Respir Crit Care Med 194(2):156-168 (2016)). Collectively, these primary tissues and cells barely constitute a convenient, reproducible model to study human respiratory pathogens. Although various cell lines, e.g. A549 and MDCK, have commonly been used to propagate influenza viruses and to study virology, they poorly recapitulate the histology of human airway epithelium. In addition, due to the low serine protease activity, most cell lines do not support the growth of the influenza viruses with monobasic HA cleavage site unless the culture medium is supplemented the exogenous serine protease, trypsin treated with N-tosyl-L-phenylalanine chloromethyl ketone (TPCK). Thus, a biologically-relevant, reproducible, and readily-available in vitro model remains desperately needed for studying biology and pathology of the human respiratory tract.

[0004] Recent advances in stem cell biology have allowed the in vitro growth of 3 dimensional (3D) organoids that recapitulate essential attributes of their counterpart-organs in vivo. These organoids can be grown from pluripotent stem cells (PSC) or tissue-resident adult stem cells (ASC) (Clevers, et al., *Cell* 165(7):1586-1597 (2016)). ASC-derived organoids consist exclusively of epithelial cells and can be generated from a variety of human organs, the first being the human gut (Sato, et al., *Gastroenterology* 141(5):1762-1772 (2011)). These human intestinal organoids represent the first model for in vitro propagation of Norovirus and has allowed the study of other viruses (Ettayebi, et al., *Science,* 353: 1387-1393 (2016); Zhou, et al., Sci. Adv. 3(11);eaao4966 (2017)). ASC-derived lung organoids have also been described (WO2016/083613).

[0005] Of note, protocols have also been established to generate lung organoids from human PSCs, embryonic lung (Chen, et al., *Nat Cell Biol* 19(5):542-549, (2017); Nikolic, et al., *Elife* 6: e26575 (2017)), embryonic stem cells and induced pluripotent stem cells (iPSC) (Konishi, et al., *Stem Cell Reports,* 6(1):18-25 (2016)).

[0006] However, there is still a need for improved methods of generating in vitro cellular systems that recapitulate the histology and functionality of mature (differentiated) human airway epithelium, for example, for use in modelling infection, particularly influenza infection. There is, in particular, a need for improved methods of differentiating ASC-derived lung organoids. Such methods would be advantageous because they do not rely on induced pluripotent stem cells, embryonic stem cells or embryonic lung. Therefore, it is the object of the present invention to provide a method of generating an in vitro cellular system that recapitulates the histology and functionality of mature human airway epithelium for use in modelling diseases, for example, influenza infection.

[0007] It is another object of the present invention to provide a method of differentiating lung organoids, preferably wherein said method does not rely on induced pluripotent stem cells, embryonic stem cells or embryonic lung.

[0008] It is another object of the present invention to provide improved in vitro differentiated lung organoids that recapitulate the histology of human airway epithelium.

[0009] It is yet another object of the present invention to provide methods for studying the biology and pathology of the human airway epithelium.